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Reviewed by: Y.M. Ioannou
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Albin B. Kocialski
Section VII, Toxicology Branch (TS-769C)

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DATA EVALUATION REPORT

Study Type: Subchronic Toxicity (Dog) Tox. Chem. No.: 716A

MRID Number: 401016-04

Test Material: Pyridate Technical

Synonyms: Lentagran; CL-11344

Study Number: TRL Study No. 043-002

Sponsor: Gilmore, Inc., Memphis, TN

Testing Facility: Toxicity Research Laboratories, Ltd.
Muskegon, MI

Title of Report: 90-Day Dog Oral Subchronic Toxicity Study

Report Issued: February 27, 1987

Conclusions:

The NOEL for general toxicity (emesis, ataxia) was found to be 20 mg/kg/day (the LDT) in both sexes of beagle dogs and the LEL for both sexes was 60 mg/kg/day (the MDT). The high dose tested, 200 mg/kg/day, exceeded the MTD (high mortality in both sexes).

Classification: Core-Guideline.

Materials and Methods:

The test compound, Pyridate Technical, a brown, oily liquid (Batch No. Khr 255 6520) with a reported chemical purity of approximately 92 percent (contaminants present were not reported by the sponsor) was used for this study. Samples of the test material were analyzed for impurities on week 5 and at the end of the study and for stability once during the study.

Male and female beagle dogs (supplied by Marshall Farms USA, Inc., North Rose, NY) 5 to 6 months old and weighing approximately 7 kg were used for this study. All dogs were immunized (by the supplier) against a number of diseases and acclimated to laboratory conditions for 3 weeks. During this period, the dogs were observed for general health and behavior and only healthy animals were used in the study.

Study Design:

A total of 16 male and 16 female beagle dogs were assigned to 4 groups/sex based on their weight as follows:

Test Group	Dose (mg/kg/day)	Number of Dogs/Group	
		Male	Female
1. Control	0	4	4
2. Pyridate (LDT)	20	4	4
3. Pyridate (MDT)	60	4	4
4. Pyridate (HDT)	200	4	4

The animals were housed individually in metal cages (with teflon-coated mesh floors) and kept in an air-conditioned room with a temperature of 71.4 + 2.6 °F, a humidity of 55.3 + 8.6 percent and a 12-hour light/dark cycle. Filtered water was available ad libitum. Purina Certified Canine diet No. 5007 was available to dogs for 1 to 2 hours the first 3 days of dosing and ad libitum thereafter. Anorectic dogs were also offered canned dog food.

The test compound was administered daily for 90 days in a gelatin capsule. The volume of the capsule was based on the body weight so that each animal received 20, 60, or 200 mg/kg/day. Control animals received an empty capsule daily.

All animals were observed daily (at 1, 2, and 3 hours after dosing) for clinical symptoms of toxicity and mortality. Body weights were recorded weekly and food consumption was measured daily. Ophthalmological examinations and electrocardiography (EKG) were performed on all test animals prior to initiation of the study and on week 13.

For hematology and clinical chemistry measurements, blood was collected from fasted dogs from the jugular vein during the pretest period and prior to dosing on weeks 2, 4, 7, and 13. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*
X	Hemoglobin (HGB)*
X	Leukocyte count (WBC)*
X	Erythrocyte count (RBC)*
X	Platelet count*
X	Prothrombin time (PT)
X	Activated Partial Thromboplastin time (APTT)

	Total plasma protein (TP)
X	Leukocyte differential count
X	Mean corpuscular HGB (MCH)
X	Mean corpuscular HGB conc. (MCHC)
	Mean corpuscular volume (MCV)

b. Clinical Chemistry

	Electrolytes:
X	Calcium*
X	Chloride*
	Magnesium*
X	Phosphorous*
X	Potassium*
X	Sodium*
	Enzymes
X	Alkaline phosphatase
X	Cholinesterase
	Creatinine phosphokinase*
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
X	Gamma-glutamyl transpeptidase (GGTP)

	Other:
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
	Cholesterol*
X	Globulins
X	Glucose*
X	Total Bilirubin*
X	Total Protein*
	Triglycerides
X	Thyroxine (T4)
X	Triiodothyronine (T4)

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

For urinalysis, urine samples were taken during the pretest period and week 13. Prior to urine collection, the dogs were intubated with approximately 100 to 150 mL of water. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrate
X	Protein*	X	Urobilinogen

c. Sacrifice and Pathology - Surviving dogs were sacrificed on day 91, 92, or 93. All animals that died and that were sacrificed on schedule, were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	Brain*
	Salivary glands*	XX	Heart*		Periph. nerve*
X	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)
X	Stomach*		Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	XX	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenals*
X	Cecum*	XX	Kidneys*	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland
X	Rectum*	XX	Testes*	XX	Parathyroids*
XX	Liver*	X	Epididymides	XX	Thyroids*
X	Gallbladder*	X	Prostate		Other
X	Pancreas*		Seminal vesicle		Bone*
	Respiratory	XX	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus	X	Skin
X	Lung			X	All gross lesions and masses

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

For histopathology, tissues were processed on a Fisher Scientific Histomatic or an AO TP/8000, embedded in paraffin, sectioned at 5 to 6 microns (brain was sectioned at 8 microns), stained with hematoxylin-eosin and examined for microscopic lesions.

Statistical Analysis:

The body weight, food consumption, organ weight, and clinical pathology data were tested for homogeneity of variance by Barlett's method (Steel and Torrie 1980). If the data were found to be homogeneous, differences between control and treatment means were tested for statistical significance by the method of Dunnett (Dunnett 1964). If the data were found not to be homogeneous, the method of Gill (modified Dunnett's) was employed (Gill 1977).

Results:

Pyridate Technical was analyzed several times throughout this study and found to have a purity ranging from 84.8 to 98.7 percent with an average purity of 94.2 percent. The authors failed to provide a list of the impurities present in samples of Pyridate Technical.

Due to the unstable nature of Pyridate (shown previously during the conduct of subchronic and chronic toxicity studies), the oral route, using gelatin capsules, was chosen for this study. The selection of the dose levels tested (20, 60, and 200 mg/kg/day) was based on a 2-week range-finding dog study where Pyridate was administered at dose levels of 1, 10, 33, 100, 200, or 400 mg/kg/day. According to the authors, no treatment-related effects were observed at dose levels 1, 10, or 33 mg/kg/day while, higher dose levels 100, 200, or 400 mg/kg/day resulted in "several marked clinical effects with neurological signs prominent," and in severely depressed body weight gains and food consumption. A dog receiving 400 mg/kg/day was sacrificed moribund on day 14. These results supported the selection of the dose levels 20, 60, and 200 mg/kg/day for the present 90-day dog study (Note: the authors submitted only a brief summary of the 2-week range-finding dog study).

Clinical Signs:

A number of compound-related clinical signs of toxicity were seen, especially in the dogs of the 60 and 200 mg/kg/day dose groups. The most pronounced clinical signs were emesis, ataxia, opisthotonos, and hypoactivity. Less predominant signs of toxicity included salivation, mydriasis, nystagmus, head swing, muscle fasiculations, and rapid and/or labored respiration and vocalization. According to the authors, all dogs of the high-dose groups (male and female) exhibited severe emesis and severe ataxia 1 to 3 hours after dosing and signs of opisthotonos, nystagmus, and mydriasis also occurred within 3 hours after dosing. Animals returned to their normal condition prior to dosing the next day for the first 20 days on study. Thereafter, some signs of toxicity persisted until dosing. Animals of the mid-dose groups exhibited (according to the authors) fewer and less severe signs of toxicity than dogs of the high-dose groups. The incidence of emesis was 8, 25, 91, and 124 times throughout the study for the control-, low-, mid-, and high-dose groups, respectively (combined incidence for both sexes), indicating clearly a dose-response relationship. The concurrent appearance of neurological signs when emesis occurred suggests that Pyridate can have an effect on the central nervous system.

Mortality:

All male dogs of the high-dose group died within 7 weeks on study (an average survival of 32.5 days) and 3 out of 4 female dogs died within 6 weeks on study (an average survival of 40 days). The high mortality rate in this dose group is attributed to Pyridate toxicity. All animals of the control-, low-, and mid-dose groups survived until termination of the study.

Ophthalmological examinations revealed that none of the surviving animals had any ocular lesions attributed to Pyridate treatment. A male dog of the high-dose group that died during the study (day 39) showed, according to the authors, signs of ocular toxicity in the form of bilateral chemosis, superficial keratitis, blepharospasm and mild aqueous flare.

Electrocardiology results (week 13, just prior to sacrifice) did not indicate any compound-related abnormalities in animals of the low- and mid-dose groups. Animals that died during the study (high-dose group male and female) were not evaluated.

Mean body weights were approximately the same between the control and the low-dose groups for both sexes throughout the study. Animals of the mid-dose groups (males and females) consistently had numerically (but not statistically significant) slightly lower mean body weights than controls at all time points examined throughout the study. Statistically significantly lower mean body weights were observed with the high-dose group males as compared to controls beginning with week 2 on study and lasting until all animals died. All animals of this group lost weight, compared to their initial body weight, throughout the study. Female dogs of the high-dose group also lost weight compared to their initial body weight until week 6 when there was only one survivor. Mean body weights for this group were consistently lower than controls and reached statistical significance level on week 4 on study.

Food consumption was numerically slightly lower in dogs of the mid-dose group (both sexes) compared to controls. Male dogs of the high-dose group had a statistically significantly decreased food consumption on weeks 2 and 3, compared to controls. No food was measured after week 3 on study since all animals were placed on supplemental canned food. Female dogs of the high-dose group consumed statistically significantly lower food quantities during week 2 on study. By week 3, 3 out of 4 dogs were placed on supplemental feed and thus for the remainder of the study food consumption measurements were derived from a single female dog.

From the hematology parameters only PCV (mid-dose males), APPT (high-dose females) and monocytes (high-dose males) measured on week 2 were found to be statistically significantly different

from the control values. These differences however, do not appear to be of any biological significance since such changes were not seen at later time points (weeks 4 or 13) and they did not appear to be dose-dependent. The platelet (PLT) count was found to be numerically higher than controls in the high-dose group males on weeks 2 and 4 on study. However, higher PLT counts were seen in the same group of animals prior to the initiation of the study (Week 1) and thus no special significance is attached to this finding.

A variety of clinical chemistry parameters measured at different time intervals were found to be statistically significantly different between treated and control groups (Table 1). Serum glutamate oxalacetate transaminase (SGOT) activities were statistically significantly lower in the high-dose females on weeks 2 and 4 on study as compared to controls. SGOT activity was numerically lower in the high-dose group males at both time points (weeks 2 and 4). Serum glutamate pyruvate transaminase (SGPT) activities were considerably lower than controls in the high-dose group of both sexes on week 2 and statistically significantly lower than controls in the mid-dose males and numerically lower in the mid- and high-dose females. Other parameters found to be statistically significantly different between treated and control groups were: Na, high-dose male and females (week 2); K, high-dose females (week 2); A/G, mid-dose females (week 2); phosphorus, high-dose males (week 4); TCO_2, high dose females (week 4) and erythrocyte cholinesterase, mid-dose males (week 13). Thyroxine (T4) and triiodothyronine (T3) values (total and free) were found to be approximately the same between treated and control groups in both sexes (week 13).

Table 1

Summary of Clinical Chemistry and Hematology Findings

Parameter	Time Point (weeks)	Dose (mg/kg/day)							
		Males				Females			
		0	20	60	200	0	20	60	200
Chemistry									
SGOT (u/L)	2	23.1	26.0	21.6	18.0	24.5	33.5	24.0	18.5*
	4	23.9	28.5	22.8	17.2	26.9	29.4	20.5	15.9**
SGPT (u/L	2	25.7	10.3	11.9	7.1	13.6	15.4	11.3	5.7
	4	17.3	10.6	12.0	3.5**	14.3	14.1	9.5**	3.8**
	13	21.3	14.6	12.9	-	16.9	16.7	9.2	6.2
Na (MEQ/L)	2	145.9	146.0	146.1	142.8*	145.8	147.2	145.4	140.8*
K (MEQ/L)	2					4.40	4.69	4.25	5.06*
A/G	2					1.17	1.2	1.39*	1.32
TCO_2 (mmol/L)	4					24.7	22.8	23.3	21.2*
PHOS (mg/dl)	4	7.46	7.00	6.76	6.05*	6.65	6.44	6.16	5.81
Hematology									
PCV (%)	2	41.5	39.0	36.2*	39.6				
APTT (sec)	2					11.2	11.2	11.0	12.9*
Mono (%)	2	6.3	8.3	9.3	12.8*				
PLT ($X10^3$/uL)	-1	182.8	205.3	183.3	254.3				
	2	170.0	165.0	175.3	271.0				
	4	181.8	169.3	152.5	304.0				
	13	146.8	120.0	184.8	-	131.3	145.0	220.5	302.0

*Statistically significantly different from control $p < 0.05$.
**Statistically significantly different from control $p < 0.01$.

Results of the urinalysis determinations did not reveal any significant changes between treated and control values.

Absolute and relative organ weights were found to be in some instances statistically significantly different between the treated and control groups. In male dogs of the low-dose group, the absolute and relative liver weight were statistically significantly higher than the controls. In female dogs, there was a dose-related trend in the increase of absolute and relative liver weight (Table 2). The absolute and relative weight of the adrenals of the mid-dose male dogs were also statistically significantly higher than the controls. Numerical increase in adrenal weight (absolute and relative) was seen in the high-dose females. Numerical decrease was observed with the absolute weight of thymus (high-dose females) and the ovaries (mid- and high-dose groups). It should be noted however, that all values for the high-dose females were obtained from a single animal.

Table 2

Organ	A[1] or R[2]	Dose (mg/kg/day)							
		Males				Females			
		0	20	60	200	0	20	60	200[3]
Liver	A	327.6	430.8*	374.5	-	301.3	353.1	359.1	402.2
	R	3.16	4.08*	3.80	-	3.26	3.78	4.16	4.62
Adrenals	A	1.22	1.30	1.54*	-	1.30	1.42	1.29	1.53
	R	11.8	12.3	15.6*	-	14.4	15.4	15.1	17.6
Thymus	A	10.3	20.6	12.0	-	10.5	12.1	13.0	4.3
	R	0.10	0.19	0.12	-	0.11	0.13	0.15	0.05
Ovaries	A					1.81	1.31	0.69	0.84

[1]Absolute organ weight (g).
[2]Relative organ weight.
[3]Only one animal.
*Statistically significantly different from control, $p < 0.05$.

Gross pathology performed on all animals (male and female) that died during the study, sacrificed at moribund condition or necropsied at the end of the study (week 13) did not reveal any significant differences in macroscopic lesions between treated and control groups. A number of lesions appeared to be of higher incidence in the high-dose group males and females compared to the other groups. However, according to the authors, many of these lesions "could be attributed to the emaciation, dehydration, and debilitation seen clinically."

Histopathological examination revealed a number of lesions associated mainly with the high-dose groups of male and female dogs. Most of these lesions were consistent with gross pathology lesions, which according to the authors, were attributed to debilitation and dehydration. Due to the randomness in the occurrence of most lesions and the lack of dose response, we do not consider them to be the direct result of Pyridate administration. These lesions are, however, reported on Table 3 for the record.

Discussion

The present study has investigated the toxicity of Pyridate to male and female beagle dogs after repeated exposure. Previous studies (subchronic and chronic) have shown that Pyridate is very unstable and at room temperatures when mixed with feed most of it is lost within 24 hours. Thus, the use of gelatin capsules was the recommended route of Pyridate administration for the present study. The dose levels selected for this study (20, 60, or 200 mg/kg/day) appeared to be the "right choice" based on the available information from the range-finding preliminary study. However, as will be discussed below, the high dose selected (200 mg/kg/day) has apparently exceeded the MTD since seven of eight dogs receiving this dose died (or sacrificed moribund) by the end of week 6 on study.

A dose-related incidence and severity of a number of clinical signs of toxicity was reported in this study. The occurrence of emesis immediately after dosing was invariably associated with a high incidence and severity of ataxia especially in the high-dose group male and female dogs suggesting that Pyridate has a toxic effect on the central nervous system. The incidence of clinical signs was comparable between the low-dose group (20 mg/kg/day) and the controls. The mid-dose group dogs (60 mg/kg/day) exhibited to a great extent, the same clinical signs as the high-dose group; however, these signs were of less frequency and severity than in the high-dose group. Thus, based on the clinical signs, the mid dose tested is considered to be the LEL while the low dose tested appears to be the NOEL for this study.

Although no mortality was reported for the control-, low-, and mid-dose groups, 100 percent mortality (4 out of 4 dogs) was observed with the high-dose group male dogs and 75 percent mortality (3 out of 4 dogs) in the high-dose group female dogs. This high mortality resulted from the direct effect of Pyridate on certain physiological functions of the animals. It appears that the statistically significant loss of weight in the animals of the high-dose group was directly related to the effect of Pyridate on appetite (and thus lower food consumption), and the effect of Pyridate on the central nervous system inducing emesis and thus higher loss of weight leading to death. The high mortality reported in the high-dose groups indicates clearly that the MTD for this study was exceeded considerably. However, the

Table 3

Summary of Histopathological Observations

Histopathological Observation	Dose (mg/kg/day)							
	Males				Females			
	0	20	60	200	0	20	60	200
Lungs with Bronchi								
- Bronchopneumonia, acute, focal	0/4[1]	0/4	0/4	1/4[2]				
- Bronchopneumonia, acute focal, severe					0/4	0/4	0/4	1/4
- Bronchopneumonia, subacute focal, slight					0/4	0/4	0/4	1/4
- Pneumonia, acute, focal	0/4	0/4	0/4	1/4				
- Edema, diffuse	0/4	0/4	0/4	1/4				
Trachea								
- Tracheitis, acute, diffuse, slight	0/4	0/4	0/4	1/4				
Liver								
- Focus of enlarged pale hepatocytes	0/4	0/4	0/4	1/4				
Spleen - Lymphocytosis	0/4	0/4	0/4	1/4				
Mesenteric lymph node - lymphocytosis	0/4	0/4	0/4	1/4				
Thymus - Lymphocytosis	0/4	0/4	0/4	1/4				
- Atrophy	0/4	0/4	0/4	1/4				
Kidney - Mineralization, renal pelvis	0/4	0/4	0/4	1/4				
- Nephritis, chronic, focal	0/4	0/4	0/4	1/4				
- Cytoplasmic vacuolization in collecting tubules	0/4	0/4	0/4	1/4				
Sciatic Nerve								
- Degenerative myelopathy, minimal	0/4	0/4	0/4	1/4	0/4	0/4	0/4	2/4
- Degenerative myelopathy, slight	0/4	0/4	0/4	1/4				
Eye - Conjunctivitis, chronic, slight	0/4	0/4	0/4	1/4				
- Ulcer, acute of cornea					0/4	0/4	0/4	1/4
- Inflammation, acute, diffuse					0/4	0/4	0/4	1/4
Bone Marrow Smear								
- Myeloid hypoplasia	0/4	0/4	0/4	1/4				
- Myeloid hyperplasia					0/4	0/4	0/4	1/4

[1] Number of dogs with lesion/total number of tissues examined.
[2] High dose groups (male and female) include animals found dead or sacrificed moribund.

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validity of the study was not affected adversely since a NOEL was shown with the low dose tested. (Note: When the present study was in progress, the sponsor contacted the Agency [Toxicology Branch] to report that male dogs of the high-dose group were dying and to receive advice as to whether the study should be terminated. Toxicology Branch advised the sponsor that the study could still be acceptable as long as a NOEL was shown with one of the two lower dose levels tested.)

Although a few hematology parameters from the treated groups were found to be statistically significantly different than controls, none of these changes appeared to be biologically significant. Similarly, several clinical chemistry parameters were found to be statistically significantly different between the treated and control groups. In most cases however, the changes appeared to be secondary to decreased food consumption (anorexia) and emesis by animals of the mid- and high-dose groups. Effects that appeared to be compound-related (i.e., increased K and decreased Na; decreased P) were not consistently present throughout the study and usually not dose-dependent.

A weak dose-related trend was observed in the increase of absolute and relative liver weight in female dogs. However, this finding might not be significant since no statistical significance was seen between the treated and control groups and this increase in liver weight was not associated with any gross/histopathological lesions. Similar results were reported with the absolute and relative weight of the adrenals in male dogs. Although the weight of the adrenals of the mid dose group was statistically significantly higher than controls, these adrenals were not in any way associated with gross/histopathological lesions. Thus, in general, changes in absolute/relative organ weights in male and female dogs did not represent a compound-related effect of toxicological significance.

Although a higher incidence of gross and microscopic lesions was seen in the high-dose groups in both sexes, these lesions did not appear to be of any biological significance since no dose-response or statistical significance was evident. We concur with the authors' conclusions that most of the gross or histopathologic lesions were secondary to debilitation, emaciation, and dehydration. The minimal to slight degenerative myelopathy of the sciatic nerve seen in dogs (2/4 males and 2/4 females) of the high-dose group appears to be a compound-related effect. It is not certain, however, that this lesion was the precursor of the neurologic abnormalities observed in the dogs of the high-dose groups.

Conclusions

The present study demonstrated that Pyridate administered to male and female dogs as gelatin capsules for 90 days causes severe toxicity and mortality at the dose level of 200 mg/kg/day (the high dose tested). Moderate overt toxicity was also observed with the administration of 60 mg/kg/day (the mid dose tested). Thus, the NOEL for general toxicity (emesis, ataxia) was considered to be 20 mg/kg/day (the lowest dose tested) in both sexes and the LEL 60 mg/kg/day in both sexes. The high dose tested, 200 mg/kg/day, was considerably higher than the MTD.

Classification: Core-Guideline.